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(54) Title: HEXITOL CONTAINING OLIGONUCLEOTIDES AND THEIR USE IN ANTISENSE STRATEGIES

(57) Abstract

The present invention relates to novel oligomers comprising 1,5-anhydro-2,3-dideoxyhexitol nucleotide analogues and deoxyribose nucleotides. The invention further relates to complex consisting ofthe novel oligomer and a complementary single-stranded or double-stranded oligonucleotide. Pharmaceutical compositions, comprising the oligomer and/or the complex and the use of both in molecular biology and genetic engineering like hybridization, isolation of nucleic acids, site specific DNA modification and mapping.

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HEXITOL CONTAINING OLIGONUCLEOTIDES AND THEIR USE IN ANTISENSE STRATEGIES

Technical field

This invention relates to oligomers comprising or containing in part 1,5-anhydrohexitol nucleotide analogues which exhibit sequence-specific binding to complementary sequences of natural oligonucleotides. This invention further relates to the chemical synthesis of these oligomers and their use in antisense strategies which comprise diagnosis, hybridization, isolation of nucleic acids, site-specific DNA modification, and therapeutics

(This application makes use of nucleoside analogues protected by an European Patent Application n° 92.201803.1, dd. 18 juni 1992, and of monomer units for chain assembly as protected by an International Patent Application n° 94202342.5, dd. 17 august 1994).

Background

The last decade new strategies have been developed for sequence specific binding of nucleic acids. One of these strategies makes use of complementary constructs to the targeted sequence according to the Watson-Crick or the Hoogsteen rule of base pairing. Modifications in the oligonucleotide structure, with the purpose of obtaining new constructs which are able to hybridize strongly with complementary DNA or RNA, could be introduced either in the base moiety either in the sugar-phosphate backbone structure. The modifications which have been integrated into an oligonucleotide have been reviewed recently (1). With the exception of a few examples [i.e. 5-methylcytosine and 5-bromocytosine (2), 2-aminoadenine (3), 7-deazaadenine (4), 5-(1-propynyl)-uracil and -cytosine (5)], incorporation of modified bases leads to less stable duplexes. The incorporation of new backbone structures into oligonucleotides generally enhances their nuclease stability and decreases their binding affinity for complementary sequences. Some sugar modifications [i.e. 2'-O-methylribonucleosides (6), carbocyclic nucleosides (7), α-nucleosides (8)] give modest enhanced target affinity. Although plenty of information is available about the duplex stability of oligonucleotides with alternative phosphate-containing linkages or phosphate-free backbones at a predetermined site (9), completely backbone modified oligonucleotides have seldom been synthesized. This is due to the unavailability of high yielding solid-support-based automated synthesis protocols for these functional groups. Two important exceptions are the morpholino-carbamate linked oligonucleotide (10) and the triple-helix forming polyamide linked acyclic nucleic acids (11). More recently oligodeoxyribonucleotide N3'→P5' phosphoramidates have been described as well, and are claimed to hybridize well to complementary oligonucleotides (12).

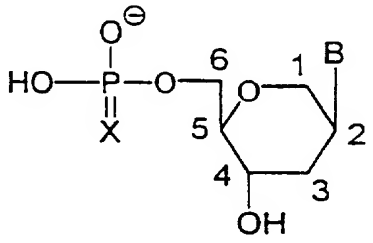
A European Patent Application n° 94202342.5 (dd. 17 august 1994) discloses the synthesis of phosphoramidite building blocks of hexitol nucleoside analogues, their assembly into oligonucleotides and the hybridization potential of this new class of oligonucleotide constructs, made up of or containing in part 1,5-anhydro-2,3-dideoxyhexitol nucleoside analogues, and phosphorylated at their 4'-end. The synthesis and antiviral activity of the afore mentioned nucleoside analogues themselves is subject of a European Patent Application n°92.201803.1, dd. 18 juni 1992. The present invention enlarges the diversity of possible constructs which can be envisaged for strong and selective hybridization with a complementary sequence as disclosed in the afore mentioned Patent Application n° 94202342.5, and further simplifies the synthesis of these constructs by elimination of the need for a 4'-terminal phosphate moiety.

Description of the invention

It is the object of the present invention to provide new oligomers, which have an improved stability and binding affinity as compared to the known oligomers.

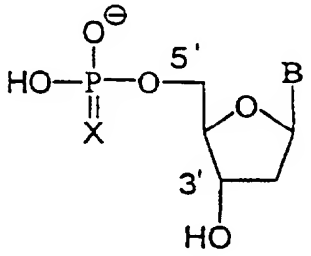
It has now been found that oligomers comprising or containing in part 1,5-anhydro-2,3-

dideoxy-D-*arabino*-hexitol nucleotide analogues as the monomer units A, wherein the hexitol is coupled via its 2-position to the heterocyclic ring of a pyrimidine or purine base, provide such improved stability and binding affinity. The monomer units A are represented by the formula I:



(I)

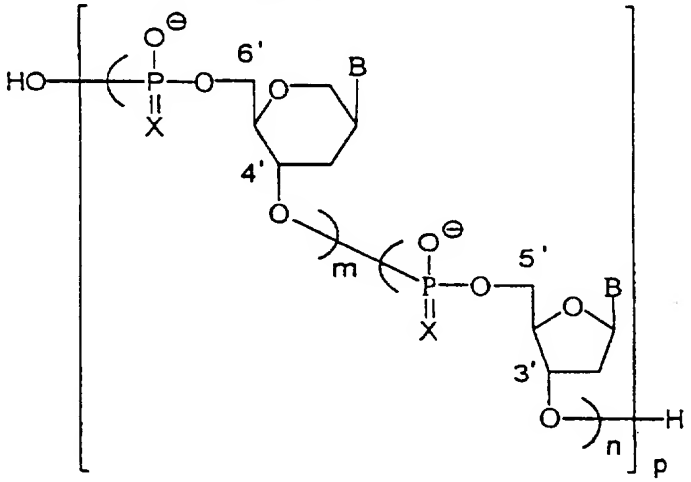
wherein B is a heterocyclic ring which is derived from a pyrimidine or purine base and X represents oxygen or sulfur. Optionally these monomer units A are combined with 2'-deoxynucleotides (monomer units B), the latter represented by formula II:



(II)

wherein B is a heterocyclic ring which is derived from a pyrimidine or purine base and X represents oxygen or sulfur.

The monomers are connected to each other through a phosphodiester bridge with formula III representing the structure of these oligomers,



(III)

wherein m, n and p are integers ;

$p \geq 2$;

each $m \geq 1$, provided that m_1 may be 0

each $n \geq 1$; provided that n_p may be 0 except when $p = 2$;

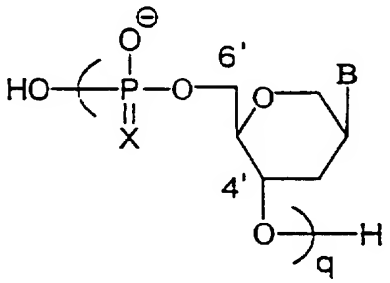
each B independently is a heterocyclic ring which is derived from a pyrimidine or purine base ;

each X independently represents oxygen or sulfur ;

the 5'-end, respectively the 6'-end, of the oligonucleotide may optionally be dephosphorylated and the 3'-end, respectively the 4'-end, may optionally be phosphorylated;

All possible salts of the compound of formula III are included in the invention.

Optionally the oligomers may be comprised solely of monomer units A (formula I) as represented by formula IV:



(IV)

wherein q is an integer with $q \geq 2$;

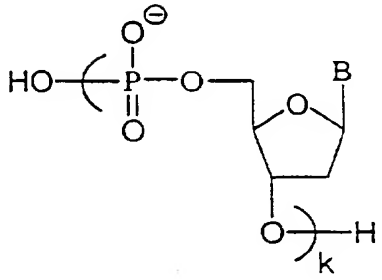
each B independently is a heterocyclic ring which is derived from a pyrimidine or purine base ;

each X independently represents oxygen or sulfur ;

the 6'-end of the oligonucleotide may optionally be dephosphorylated;

and all possible salts thereof.

The oligomers of formula III and IV are new compounds. They display a certain similarity with oligonucleotides consisting of the natural 2'-deoxynucleosides represented by formula V



(V)

wherein k is an integer and wherein each B independently is a heterocyclic ring which is derived from a pyrimidine or purine base, and wherein the 5'-end of the oligonucleotide may optionally be dephosphorylated.

According to the invention it has been found that the oligomers of formula III and IV and their respective salts exhibit sequence specific binding to natural oligonucleotides represented by formula V or its 5'-dephosphorylated congener. A new class of hybridons or sequence-specific binding polymers has therefore been found.

Oligomers of formula III or IV may form a complex with another oligomer of formula III or IV, or they may form a complex with a complementary single-stranded or double-stranded natural oligonucleotide. Each strand may be of the same or different length.

The fact that sequence-specific binding is found for the oligomers of formula III as well as IV with the compounds of formula V, must be deemed surprising and has been discussed and explained in detail in the afore mentioned Patent Application n° 94202342.5. Enlarging the furan ring of furanose compounds to a pyran ring did not yield oligomers capable of binding natural oligonucleotides (compare ref. 13-14). Thus, the effect of enlarging the pentofuranosyl ring to a 1,5-anhydrohexitol ring could not be anticipated.

The compounds according to the invention are therefore oligomers comprising or containing partially nucleotide analogues wherein a 1,5-anhydro-2,3-dideoxy-D-hexitol is coupled via its 2-position according to an *arabino*-configuration to the heterocyclic ring of a pyrimidine or purine base. The oligomers consist of the above mentioned nucleotide analogues connected to each other as phosphate diesters or thiophosphate diesters. The oligomers can be represented either by the formula III or IV, wherein m, n, p, q, B and X have the above stated designations. The oligomers can be exclusively composed of the hexitol nucleotide analogues of the formula I (yielding oligomers of formula IV) or can have natural 2'-deoxynucleotides interspersed or at the end of the molecule (yielding oligomers of formula III). The hexitol has the (D)-configuration and the stereochemistry of both substituents is according to an (S) configuration.

When group B is derived from a pyrimidine base it can be either cytosine, 5-methylcytosine, uracil or thymine. When B is derived from a purine base it can be either adenine, guanine, 2,6-diaminopurine, hypoxanthine or xanthine.

The invention also relates to the chemical synthesis of these oligomers and their salts and to their use in antisense strategies which comprise diagnosis, hybridization, isolation of nucleic acids, site-specific DNA modification and therapeutics.

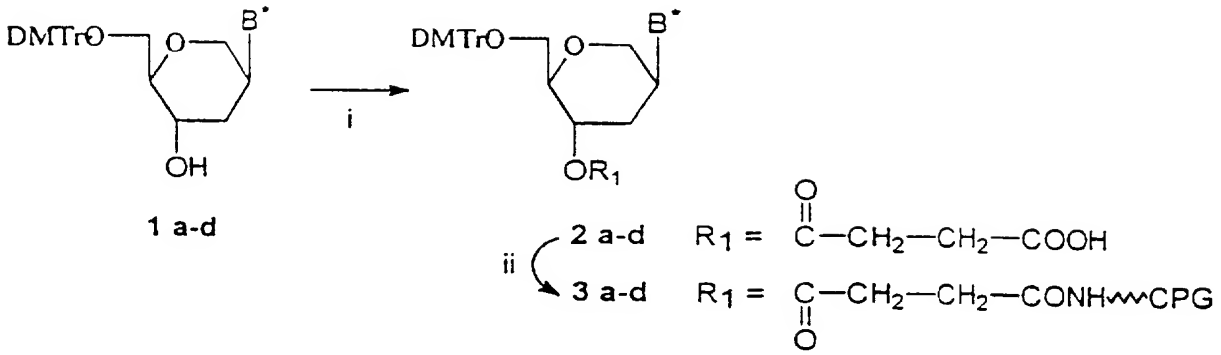
Chemical synthesis

The nucleoside analogues, monomer components of the present invention can be prepared in different ways and one of the preparation methods is subject of european patent application n° 92.201803.1. These syntheses have been described likewise (15). Assembly of the monomers into an oligomer follows the classical schemes and can be done either by standard phosphoramidite chemistry (compare ref. 16) or by H-phosphonate chemistry (compare ref. 17). All procedures are conveniently carried out on an automated DNA synthesizer as for standard oligonucleotide synthesis. For these standard conditions also compare e.g. ref. 18. The preferred method is the phosphoramidite method making use of the phosphoramidites of the hexitol nucleoside analogues as the incoming building blocks for assembly in the "6'-direction". The method of preparation for these phosphoramidite building units is subject of european patent application n° 94202342.5.

Synthesis of the afore mentioned oligonucleotide constructs of formula III makes use of standard solid supports (e.g. controlled pore glass) functionalized with an appropriately protected 2'-deoxynucleoside. Following standard procedures and making use of well-known phosphoramidite monomers of 2'-deoxynucleosides on one hand and phosphoramidite building units of 1,5-anhydro-2,3-dideoxyhexitol analogues as subject of european patent application n° 94202342.5 on the other hand, assembly of any composition of formula III can be obtained. Following removal of the dimethoxytrityl protecting group of the last coupled 1,5-anhydro-2,3-dideoxyhexitol analogue or 2'-deoxynucleotide, the primary hydroxyl group optionally can be phosphorylated by well-known commercially available reagents (e.g. phosphorylation reagent of Glen Research) or can be left intact.

Synthesis of the afore mentioned oligonucleotide constructs of formula IV can be done starting from solid supports functionalized with an appropriately protected 1,5-anhydro-2,3-dideoxyhexitol analogue. The synthesis of such supports is outlined below. Following standard solid phase procedures and using the afore mentioned phosphoramidite building units of 1,5-anhydro-2,3-dideoxyhexitol analogues, constructs of any composition of formula IV can be obtained. Following removal of the dimethoxytrityl protecting group of the last coupled 1,5-anhydro-2,3-dideoxyhexitol analogue, the primary hydroxyl group optionally can be phosphorylated by well-known commercially available reagents (e.g. phosphorylation reagent of Glen Research) or can be left intact.

The assembly of the constructs of formula III and IV most conveniently is performed on an automated DNA synthesizer using phosphoramidite chemistry. Alternatively different chemistry can be used like the phosphonate strategy, and assembly can be done in solution with either of both strategies. This is exemplified by synthesis of a dimer in solution by the phosphonate strategy as described further.



a: B* = thymin-1-yl

b: B* = N^6-benzoyladen-9-yl

c: B* = N^2-isobutyrylguanin-9-yl

d: B* = N^4-benzoylcytosin-1-yl

i: DMAP, succinic anhydride, pyridine; ii: pre-activated LCAA-CPG, DMAP, Et_3N, 1-(3-diethylaminopropyl)-3-ethylcarbodiimide · HCl, pyridine

Solid supports containing a 1,5-anhydrohexitol analogue can be prepared by succinylation of the compounds **1a-d** yielding **2a-d**, which can be coupled to the amino function of either long chain alkylamino controlled pore glass (CCAA-CPG) or a suitable amino functionalized polystyrene (e.g. Tentagel^R - RAPP Polymere) making use of a carbodiimide and yielding **3a-d** (for functionalization of supports compare ref. 19).

After assembly, the obtained oligonucleotides are cleaved from the support and deprotected by ammonia treatment for 16 h at 55°C. Purification of the obtained oligomers of the above stated formula's III or IV can be accomplished in several ways (compare e.g. ref. 20). The preferred method is purification by anion-exchange FPLC at a basic pH of 12 to disrupt all possible secondary structures (compare e.g. ref. 13). Desalting is done by simple gel filtration techniques followed by lyophilization. All acceptable salts can be prepared in a conventional manner.

The compounds according to the invention as well as their chemical synthesis and the preparation of the starting materials are further illustrated in the following examples, which are not however intended to limit the invention.

Abbreviations : LSIMS liquid secondary ion mass spectrometry, Thgly thioglycerol, TEA triethylamine, TEAB triethylammonium carbonate, TEAA triethylammonium acetate.

Synthesis of the 1,5-anhydro-2,3-dideoxy-2-substituted-D-*arabino*-hexitol nucleoside analogues and of their 4,6-O-benzylidene protected derivatives has been described by Verheggen et al. (15). Base protection, dimethoxytritylation and succinylation of these hexitol nucleoside analogues likewise has been described in patent application n° EU 94202342.5.

1,5-anhydro-6-*O*-monomethoxytrityl-2-(thymin-1-yl)-2,3-dideoxy-D-arabinohexitol-4-yl-hydrogenphosphonate, triethylammonium salt (6a)

To 270 µL (3 mmol) of phosphorus trichloride in 30 mL of anhydrous CH_2Cl_2 cooled on an icebath, was added 3.3 mL (30 mmol) of N-methylmorpholine and 690 mg (10 mmol) of 1,2,4-triazole. The mixture was stirred for 30 min at room temperature, subsequently cooled again, and 320 mg (0.6 mmol) of 1,5-anhydro-6-*O*-monomethoxytrityl-2-(thymin-1-yl)-2,3-dideoxy-D-arabinohexitol (**4a**) dissolved in 10 mL of CH_2Cl_2 was added dropwise over 15 min. The mixture was stirred for 10 min. more at room temperature and poured into 25 mL of 1 M TEAB buffer. After separation of both layers, the aqueous phase was extracted once more with CH_2Cl_2 and the organics were dried and purified by flash chromatography on 20 g of silica gel (gradient of CH_2Cl_2-TEA 99:1 to CH_2Cl_2-TEA-MeOH 89:1:10). Product containing fractions were washed once with 25 mL of 1 M TEAB buffer and dried, affording 344 mg (0.495 mmol, 82%) of the title product **6a** as a white foam.

LSIMS (ThGly doped with NaOAc) m/z 659 ($M+3Na^+$, 1), 637 ($M+2Na^+$, 2), 273 ($MMTr^+$, 100)

^1H NMR ($CDCl_3$): δ 1.27 (t, 9H, J=7Hz, $CH_2\underline{CH_3}$), 1.74 (s, 3H, CH_3), 1.94-2.11 (m, 1H, H-3'$_{ax}$), 2.61 (dm, 1H, H-3"$_{eq}$), 3.00 (q, 6H, $\underline{CH_2}CH_3$), 3.34 (dd, 1H, J= 4.5 and 10.4Hz, H-5'), 3.45-3.60 (m, 2H, H-6', H-6"), 3.79 (s, 3H, OCH_3), 3.89 (dd, 1H, J= 3.4 and 13.4Hz, H-1'$_{ax}$), 4.16 (d, 1H, J= 13.6Hz, H-1'$_{eq}$), 4.57 (m, 1H, H-4'), 4.73 (m, 1H, H-2'), 6.66 (d, 1H, J=625 Hz, P-H), 6.83 (d, J = 8.7, 2H, aromatic H), 7.15-7.55 (m, 12H, aromatic H), 8.05 (s, 1H, H-6), 9.50 (br s, 1H, NH) ppm.

^{13}C NMR ($CDCl_3$) δ 8.5 ($CH_2\underline{CH_3}$), 12.7 (CH_3), 35.2 (C-3'), 45.3 ($\underline{CH_2}CH_3$), 50.4 (C-2'), 55.1 (OCH_3), 62.7 (C-6'), 64.2 (C-4', d, J=3.8Hz), 68.4 (C-1'), 80.4 (C-5', d, J=8Hz), 86.2 (Ph_3C), 110.5 (C-5), 138.5 (C-6), 151.2 (C-2), 163.9 (C-4), 113.0, 126.8, 127.4, 127.7, 130.4, 135.5, 144.3, 158.4 (aromatic C) ppm.

1,5-anhydro-6-*O*-monomethoxytrityl-2-(N^6-benzoyladenin-9-yl)-2,3-dideoxy-D-arabinohexitol-4-yl-hydrogenphosphonate, triethylammonium salt (6b)

To 315 µL (3.5 mmol) of phosphorus trichloride in 30 mL of anhydrous CH_2Cl_2 cooled on an icebath, was added 3.85 mL (35 mmol) of N-methylmorpholine and 830 mg (12 mmol) of 1,2,4-triazole. The mixture was stirred for 30 min at room temperature, subsequently cooled again, and 450 mg (0.7 mmol) of 1,5-anhydro-6-*O*-monomethoxytrityl-2-(N^6-benzoyladenin-9-yl)-2,3-dideoxy-D-arabinohexitol (**4b**) dissolved in 10 mL of CH_2Cl_2 was added dropwise over 15 min. The mixture was stirred for 10 min. more at room temperature and poured into 25 mL of 1 M TEAB buffer. After separation of both layers, the aqueous phase was extracted once more with CH_2Cl_2 and the organics were dried and purified by flash chromatography on 20 g of silica gel (gradient of CH_2Cl_2-TEA 99:1 to CH_2Cl_2-TEA-MeOH 89:1:10). Product containing fractions were washed once with 25 mL of 1 M TEAB buffer and dried, affording

516 mg (0.64 mmol, 92%) of the title product **6b** as a white foam.
LSIMS (ThGly doped with NaOAc) m/z 722 ($M+3Na^+$, 2), 750 ($M+2Na^+$, 8), 273 ($MMTr^+$, 100)
^1H NMR ($CDCl_3$): δ 1.22 (t, 9H, J=7Hz, $CH_2\underline{CH_3}$), 2.10-2.29 (ddd, 1H, H-3'$_{ax}$), 2.82 (dm, 1H, H-3"$_{eq}$), 2.88 (q, 6H, $\underline{CH_2}CH_3$), 3.35 (dd, 1H, J= 4.7 and 10.3Hz, H-5'), 3.56 (dd, 1H, J=1.8 and 10.3Hz, H-6'), 3.62-3.74 (m, 1H, H-6"), 3.79 (s, 3H, OCH_3), 4.03 (dd, 1H, J= 2.4 and 13.0Hz, H-1'$_{ax}$), 4.37 (d, 1H, J= 13.0Hz, H-1'$_{eq}$), 4.46 (m, 1H, H-4'), 5.04 (m, 1H, H-2'), 6.56 (d, 1H, J=625.3Hz, P-H), 6.84 (d, J = 8.7, 2H, aromatic H), 7.15-7.65 (m, 15H, aromatic H), 8.05 (d, 2H, aromatic H), 8.73 (s, 1H) and 8.79 (s, 1H) (H-2, H-8), 9.37 (br s, 1H, NH) ppm.
^{13}C NMR ($CDCl_3$) δ 8.4 ($CH_2\underline{CH_3}$), 35.8 (C-3'), 45.2 ($\underline{CH_2}CH_3$), 50.6 (C-2'), 55.1 (OCH_3), 62.7 (C-6'), 64.3 (C-4', d, J=5.5Hz), 69.0 (C-1'), 80.7 (C-5', d, J=9.3Hz), 86.3 (Ph_3C), 122.4 (C-5), 142.7 (C-8), 149.5 (C-4), 151.7, 152.3 (C-2, C-6), 166.8 (CO) ppm + aromatic signals.

1,5-anhydro-4-*O*-benzoyl-2-(thymin-1-yl)-2,3-dideoxy-D-arabinohexitol (5a)

An amount of 225 mg (0.42 mmol) 1,5-anhydro-6-*O*-monomethoxytrityl-2-(thymin-1-yl)-2,3-dideoxy-D-arabinohexitol (**4a**) was dissolved in 10 mL of anhydrous pyridine and treated with 100 µL (0.84 mmol) of benzoyl chloride overnight at room temperature. The mixture was concentrated, poured into 25 mL CH_2Cl_2 and washed twice with a 5% $NaHCO_3$ solution. The organic phase was dried, evaporated and coevaporated twice with toluene. The obtained foam was treated with 40 mL of a 80% aqueous acetic acid solution for 1 h at 60°C. Evaporation and coevaporation with toluene left an oil which was purified by flash chromatography on 20 g of silica gel (gradient of CH_2Cl_2 to CH_2Cl_2-MeOH 94:6). Besides some 4'-*O*, N^3-dibenzoylated product, 108 mg (0.3 mmol, 71%) of the title product **5a** was isolated as a white foam.
LSIMS (ThGly) m/z 383 ($M + Na^+$, 10), 361 (MH^+, 20), 239 (MH^+ - BzOH, 50), 127 (BH_2^+), 105 (C_7H_5O, 100).

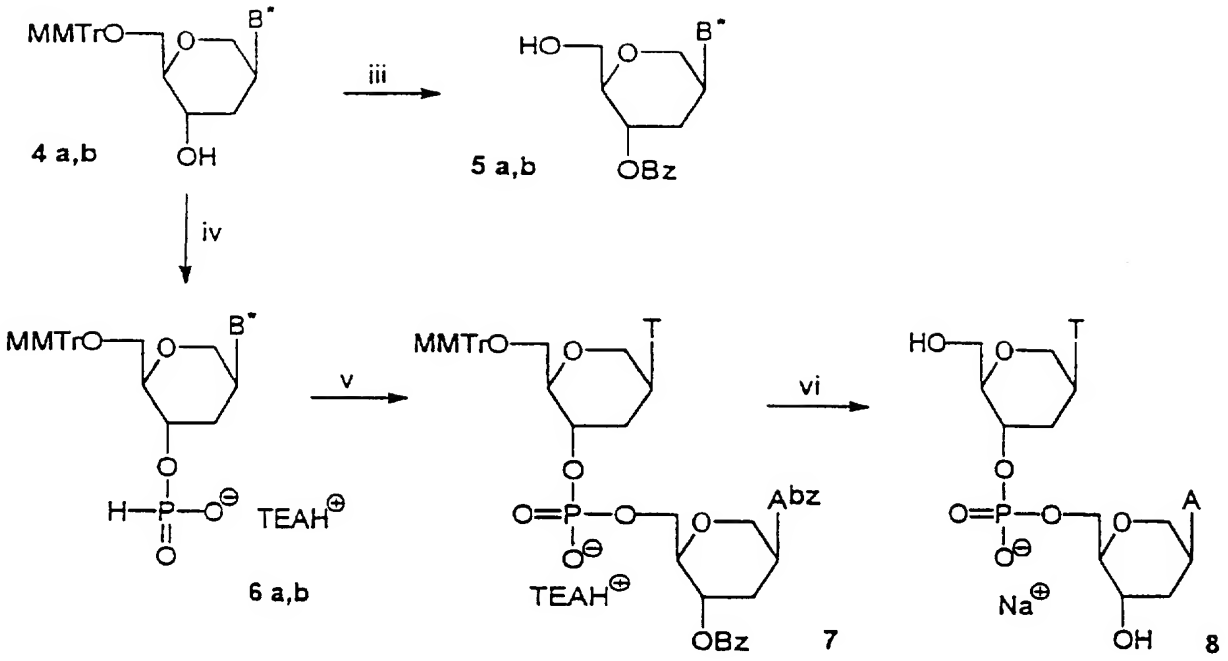
1,5-anhydro-4-*O*-benzoyl-2-(N^6-benzoyladenin-9-yl)-2,3-dideoxy-D-arabinohexitol (5b)

An amount of 257 mg (0.4 mmol) of **4b** was coevaporated with pyridine and subsequently dissolved in 20 mL of anhydrous pyridine after which 110 µL (0.8 mmol) of benzoyl chloride was added. The mixture was stirred overnight at room temperature and quenched with 2 mL of methanol. After addition of some $NaHCO_3$, the mixture was concentrated and partitioned between CH_2Cl_2 and 5% of aq. $NaHCO_3$. The organic phase was washed once more with 75 mL of aq. $NaHCO_3$ and dried on Na_2SO_4. Evaporation left an oil which was coevaporated with toluene. The foam was treated with 40 mL of 80% aq. HOAc and left at RT for 1 h. Evaporation gave 400 mg of a light brown foam which was purified on silica gel with a methanol gradient in CH_2Cl_2 (100 to 96:4). The product containing fractions were pooled

affording 86 mg (0.18 mmol, 45%) of the title product 5b as a white powder.
LSIMS (ThGly) m/z 474 (MH^+, 25), 240 (BH_2^+, 7).
^1H NMR ($CDCl_3$): δ 2.21-2.39 (ddd, 1H, H-3'$_{ax}$), 2.84-2.98 (dm, J=13.5Hz, 1H, H-3"$_{eq}$), 3.64-3.91 (m, 3H, H-5', H-6', H-6"), 4.13 (dd, 1H, J= 2.6 and 13.1Hz, H-1'$_{ax}$), 4.52 (dm, 1H, J= 12.5Hz, H-1'$_{eq}$), 5.07-5.14 (m, 1H, H-2'), 5.18-5.33 (m, 1H, H-4'), 7.35-7.65 (m, 6H, aromatic H), 7.91-8.08 (m, 4H, aromatic H), 8.70 (s, 1H) and 8.80 (s, 1H) (H-2, H-8), 9.63 (br s, 1H, NH) ppm.
^{13}C NMR ($CDCl_3$) δ 33.1 (C-3'), 50.7 (C-2'), 61.4 (C-6'), 64.4 (C-4'), 69.1 (C-1'), 80.5 (C-5'), 123.2 (C-5), 142.0 (C-8), 149.6 (C-4), 151.7 (C-6), 152.5 (C-2), 164.8 and 165.4 (2 x CO) ppm + aromatic signals.

[1,5-anhydro-2-(thymin-1-yl)-2,3-dideoxy-D-arabinohexitol]-(4-6)-[1,5-anhydro-2-(adenin-9-yl)-2,3-dideoxy-D-arabinohexitol]-phosphate, sodium salt (8)

A portion of 86 mg (0.18 mmol) of **5b** and 145 mg (0.21 mmol) of the thymine phosphonate **6a** were coevaporated twice with anhydrous pyridine, and dissolved in a 6 mL mixture of anhydrous pyridine and acetonitrile. Then 1 mL (0.5 mmol) of a dilution of pivaloyl chloride in pyridine was added and the reaction mixture was stirred for 10 min at room temperature under a nitrogen atmosphere. Subsequently 2 mL of a 4% I_2 (w/v) solution in pyridine-water (96:4) was added and stirring continued for another 10 min. at room temperature. The mixture was diluted with 100 mL of CH_2Cl_2 and washed with 50 mL of 10% aqueous $Na_2S_2O_3$ and with 50 mL of 1 M TEAB. The organic phase was dried, evaporated and purified by column chromatography on 20 g of silica gel (gradient of CH_2Cl_2-TEA 99:1 to CH_2Cl_2-TEA-MeOH 93:1:6) affording 200 mg (0.17 mmol, 95%) of the fully protected dimer.
LSIMS (ThGly doped with NaOAc) m/z 1108 ($M-2Na^+$, 2), 273 ($MMTr^+$, 100)
The white foam obtained was dissolved in 32 mL of a 3:1 mixture of conc. ammonia and ethanol and heated overnight at 40°C. The mixture was evaporated and coevaporated with 1,4-dioxane. The residue was dissolved in 20 mL of 80% aqueous acetic acid and heated at 60 °C for 1 h. After evaporation, the residue was partitioned between 10 mL of 10 mM TEAA solution and 10 mL of ether. The aqueous phase was washed 3 times more with 10 mL of diethylether and concentrated and purified by reverse phase HPLC on a polystyrene-divinyl-benzene support (PLRP-S, 250 x 9 mm) with an acetonitrile gradient in 0.1 M TEAA. Product containing fractions were pooled and the TEAA salt was exchanged for the sodium salt by chromatography on a Dowex 50X8-200 cation exchange resin under its sodium form. Lyophylization afforded 52 mg (85 μmol, 47% overall) as a white voluminous powder.
LSIMS (ThGly) m/z 628 (M sodium salt + Na^+, 30), 606 (M sodium salt + H^+, 4).



a: B* = thymin-1-yl b: B* = N^6-benzoyladen-9-yl

iii: benzoyl chloride, pyridine; 80% HOAc; iv: PCl_3, N-methylmorpholine, triazole; TEAB buffer; v: pivaloyl chloride, pyridine-acetonitrile; iodine; TEAB buffer; vi: ammonia-ethanol; 80% HOAc; RP-HPLC; Dowex 50X8-200 (Na^+ form)

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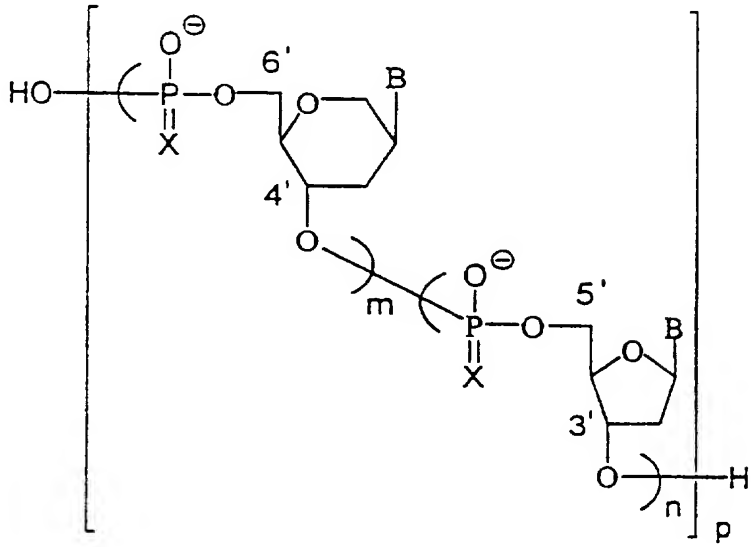
Claims

1. An oligomer comprising 1,5-anhydro-2,3-dideoxyhexitol nucleotide analogues and deoxyribose nucleotides having the general formula :



wherein m, n and p are integers ;

$p \geq 2$;

each $m \geq 1$, provided that m_1 may be 0

each $n \geq 1$; provided that n_p may be 0 except when $p = 2$;

each B independently is a heterocyclic ring which is derived from a pyrimidine or purine base ;

each X independently represents oxygen or sulfur ;

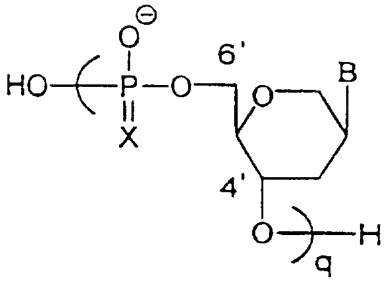
the 5'-end, respectively the 6'-end, of the oligonucleotide may optionally be dephosphorylated and the 3'-end, respectively the 4'-end, may optionally be phosphorylated;

and all possible salt forms thereof.

2. An oligomer according to claim 1 wherein $p > 2$.

3. An oligomer according to claim 1 wherein $m_1 \geq 1$ and $n_p = 0$ such that there is at least one 1,5-anhydro-2,3-dideoxyhexitol moiety at each end of the oligomer.

4. An oligomer comprising 1,5-anhydro-2,3-deoxyhexitol nucleotide analogues having the general formula :



wherein q is an integer with $q \geq 2$;
each B independently is a heterocyclic ring which is derived from a pyrimidine or purine base ;
each X independently represents oxygen or sulfur ;
the 6'-end of the oligonucleotide may optionally be dephosphorylated;
and all possible salt forms thereof.

5. An oligomer as claimed in any one of claims 1 to 4, wherein the heterocyclic ring B, when derived from a pyrimidine base, is selected from the group consisting of cytosine, 5-methylcytosine, uracil or thymine.

6. An oligomer as claimed in any one of claims 1 to 4, wherein the heterocyclic ring B, when derived from a purine base, is selected from the group consisting of adenine, guanine, 2,6-diaminopurine, hypoxanthine or xanthine.

7. An oligomer as claimed in any one of claims 1 to 4, wherein the heterocyclic ring B is cytosine, thymine, adenine or guanine.

8. An oligomer as claimed in any one of claims 1 to 7 wherein the 1,5-anhydro-2,3-dideoxyhexitol nucleotide analogues have the (D) configuration and the stereochemistry of both substituents is according to the (S) configuration.

9. A complex comprising a first oligomer as claimed in any one of claims 1 to 8 and either a complementary single-stranded or double-stranded natural oligonucleotide or a self-complementary second oligomer as claimed in any one of claims 1 to 9, wherein each of the strands in the complex may be of the same or of a different length.

10. An oligomer as claimed in any one of claims 1 to 8 or a complex according to claim 9 for use as a medicine.

11. A pharmaceutical composition comprising as an active ingredient a therapeutically effective amount of an oligomer as claimed in any one of claims 1 to 8 or a complex as claimed in claim 9, and a pharmaceutically acceptable carier.

12. A process of preparing a pharmaceutical composition as claimed in claim 11, characterized in that a therapeutically effective amount of an oligomer as claimed in any one of claims 1

to 8 or a complex as claimed in claim 9, is intimately mixed with a pharmaceutically acceptable carrier.

13. The use of an oligomer as claimed in any one of claims 1 to 8 or of a complex as claimed in claim 9, in molecular biology and genetic engineering.

14. Uses according to claim 13 comprising hybridization, isolation of nucleic acids (isolation of DNA and RNA fragments), site-specific DNA modification, mapping.

INTERNATIONAL SEARCH REPORT

International Application No
PC., EP 97/00762

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H21/00 C12Q1/68 C07F9/6561 C07F9/6558 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H C12Q C07F A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANGEWANDTE CHEMIE INTERNATIONAL EDITION IN ENGLISH, vol. 34, no. 12, 1995, pages 1338-1339, XP000605755 A.V.AERSCHOT ET AL.: "1,5-Anhydrohexitol Nucleic Acids, a New Promising Antisense Construct." see the whole document ---	1-14
X	ACS SYMPOSIUM SERIES 580 CARBOHYDRATE MODIFICATIONS IN ANTISENSE RESEARCH, 1994, pages 80-99, XP000605743 P.HERDEWIJN ET AL.: "Hexopyranosyl-Like Oligonucleotides." see the whole document ---	1-5,7, 10-14
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 April 1997	22.04.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Scott, J

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/00762

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NUCLEIC ACIDS RESEARCH, vol. 20, no. 18, 1992, OXFORD GB, pages 4711-4716, XP000605720 K.AUGUSTYNS ET AL.: "Incorporation of Hexose Nucleoside Analogues into Oligonucleotides : Synthesis, Base-pairing Properties and Enzymatic Activity." see the whole document	1-5,7, 10-14
A	NUCLEIC ACIDS RESEARCH, vol. 21, no. 20, 1993, OXFORD GB, pages 4670-4676, XP000605721 K.AUGUSTYNS ET AL.: "Hybridization Specificity, Enzymatic Activity and Biological (Ha-ras) Activity of Oligonucleotides Containing 2,4-dideoxy-beta-D-erythro-hexopyranosyl Nucleosides." see the whole document	1
A	US 5 314 893 A (TINO ET AL.) 24 May 1994 see abstract	1
A	WO 93 25565 A (STICHTING REGA VZW) 23 December 1993 see abstract	1
P,X	WO 96 05213 A (STICHTING REGA VZW) 22 February 1996 cited in the application see the whole document	1-14

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 97/00762

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 13 and 14 are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte· ·onal Application No
PC1/EP 97/00762

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5314893 A	24-05-94	AU 672845 B	17-10-96
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		EP 0608809 A	03-08-94
		JP 6271574 A	27-09-94
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		US 5607922 A	04-03-97
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